buffer, pH 8.0 (solution 3), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25- milliliter quantities of solution 3. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 3. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 3 to the reference concentration of 1.0 microgram of neomycin per milliliter (estimated).

(iii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, except add to each concentration of the polymyxin B standard response line a quantity of neomycin to yield the same concentration of neomycin as that present when the sample is diluted to contain 10 units of polymyxin B per milliliter. Prepare the sample for assay as follows:

(a) If the ointment is not water miscible. Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of solution 6. Combine the buffer extractives in a suitable volumetric flask and dilute to volume with solution 6. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(b) If the ointment is water miscible. Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

[42 FR 27235, May 27, 1977, as amended at 55 FR 50173, Dec. 5, 1990]

§448.513d Bacitracin zinc-polymyxin B sulfate topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate topical powder contains bacitracin zinc and polymyxin B sulfate in a suitable and harmless base. Each gram contains 500 units of bacitracin and 10,000 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 7.0 percent. It contains not more than an average of 10 microoganisms per gram. The bacitracin zinc used conforms to the standards prescribed by §448.13(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1).

(2) *Labeling*—(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(*c*) An expiration date that conforms to the requirements prescribed by §432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of test and assays on:

(*a*) The bacitracin zinc used in making the batch for potency, loss on drying, pH, zinc content, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, and identity.

(*c*) The batch for bacitracin content, polymyxin B content, moisture, and a microorganism count.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The bacitracin zinc used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 1.0 gram.

(*c*) The batch: A minimum of 12 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Bacitracin content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Wash an accurately weighed sample (usually 2 grams) into a 100-milliliter volumetric flask with 0.01N hydrochloric acid. Dilute to volume with 0.01N hydrochloric acid. Further dilute an aliquot with solution 1 to the reference concentration of 1.0 unit of bacitracin per milliliter (estimated).

NOTE: The final sample solution must contain the same amount of hydrochloric acid as the reference concentration of the working standard.

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed representative portion of the sample (usually 1 gram) in 20 milliliters of sterile distilled water. Wash into an appropriate-sized volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6). Further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Microorganism count*—(i) *Conduct of test for bacteria.* Using approximately 200 milligrams of powder from each of five separate immediate containers, proceed as directed in §436.20(e)(1) of this chapter, except after the three washings transfer the entire filter membrane to the surface of medium N as described in §436.20(c)(14) of this chapter. Incubate the plate for 7 days at 30° C. to 32° C. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

(ii) *Conduct of test for molds and yeasts.* Proceed as directed in §436.20(e)(1) of this chapter, using approximately 200 milligrams from each of the five containers tested, except transfer the entire filter membrane to the surface of medium N as described in §436.20(c)(14) of this chapter, and incubate at 22° C. to 25° C. for 7 days. Count the number of colonies appearing on the filter pad and calculate therefrom the number of viable microorganisms per gram of powder.

[42 FR 27236, May 27, 1977, as amended at 50 FR 15110, Apr. 17, 1985; 55 FR 11584, Mar. 29, 1990]

§448.513e Bacitracin zinc-polymyxin B sulfate topical aerosol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Bacitracin zinc-polymyxin B sulfate topical aerosol is bacitarcin zinc, polymyxin B sulfate in a suitable and harmless vehicle, packaged in a pressurized container with suitable and harmless inert gases. Each gram contains 120 units of bacitracin and 2,350 units of polymyxin B. Its bacitracin content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of bacitracin that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 0.5 percent. It contains not more than an average of 10 microorganisms per container. The bacitracin zinc used conforms to the standards prescribed by §448.13(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30(a)(1).